



UNIVERSITI PUTRA MALAYSIA

**AN EXAMINATION OF EMBRYOGENIC AND NON-EMBRYOGENIC
CULTURES OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.)**

ONG LI MEI

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CULTURES OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.)**

By

ONG LI MEI

**This is submitted in Fulfilment of the Requirement for the
Degree of Doctor of Philosophy in the Faculty of
Food Science and Biotechnology
Universiti Putra Malaysia**

March 2001



**Specially Dedicated
To the
ONGs and ALWIs**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy.

**AN EXAMINATION OF EMBRYOGENIC AND NON-EMBRYOGENIC
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March 2001

Chairman : Associate Professor Dr. K. Harikrishna, Ph.D

Faculty : Food Science and Biotechnology

Somatic embryogenesis of crop plants such as oil palm has generated considerable research interest. However, the main obstacle that hinders the development of an economically viable propagation system is the low frequency of embryogenesis. Currently, most local tissue culture laboratories are reporting embryogenesis rates of approximately 6%. Due to the lack of knowledge about oil palm somatic embryogenesis, it would be difficult to understand or even to attempt to improve the process. Hence, this study has been tailored to understand the fundamental processes that could occur during embryogenesis by analyzing differences between embryogenic (EC) and non-embryogenic (NEC) *in vitro* cultures of oil palm.

The initial studies concentrated on elucidating the differences found between EC and NEC at the microscopical level. Proembryo (PE) structures were predominantly found in ECs. The phenomenon of isolation of cells, as a prerequisite to embryogenesis, was observed in the formation of PEs. It was hypothesized that the surrounding cells at the periphery of each PE structure could have gone through programmed cell death (PCD) hence creating the condition of 'isolation of cells'.

The hypothesis that PCD could play an important role in the embryogenesis process was further supported by studies carried out at the physiological and molecular level. ECs were found to be metabolically more active than NECs, thus indicating that ECs would need an efficient system to overcome the accumulation of reactive oxygen species (ROS), a toxic byproduct of aerobic metabolism. With the isolation of the embryogenic tissue specific OPEm1, which encodes for an antioxidant known as peroxiredoxin, it is believed that it functions by protecting the proembryos from being damaged by the ROS but killing the cells surrounding them.

OPEm1 represents the first peroxiredoxin to be isolated from a palm and has potential to be exploited as a molecular marker for embryogenic potential of *in vitro* cultures. In addition to this, with the knowledge of the physiological state of embryogenic and non-embryogenic cultures, a non-destructive method for the detection of embryogenic potential can now be devised by taking advantage of the reaction mechanism of oxidative dyes in culture media.

Besides this, attempts were also made to isolate other embryogenic related genes by means of a rapid cloning method for differentially expressed cDNAs. This technique is better known as Suppression Subtractive Hybridization. Out of a total 595 clones screened, only 66 were found to be embryogenic specific. Amongst these clones, one of them was characterized and shown to be closely related to a class IV chitinase EP3. This clone was designated as OPSSH1. There is some evidence to suggest from the northern analysis study, that different subsets of class IV endochitinase EP3 were being detected, as two differently sized transcripts were observed. It is possible that they encode proteins that have differing functions. However, due to the

generally short fragments being produced through this technique, it is still too early to propose a functional role for endochitinase(s) in the oil palm system.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**KAJIAN KE ATAS KULTUR EMBRIOGENIK DAN TAK-EMBRIOGENIK
KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)**

Oleh

ONG LI MEI

Mac 2001

Pengerusi : Profesor Madya Dr. K. Harikrishna, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Embriogenesis somatik tumbuh-tumbuhan, seperti kelapa sawit, telah menjana minat yang mendalam terhadap penyelidikan. Walau bagaimanapun, batu penghalang bagi perkembangan sesuatu sistem pembiakan yang berdaya maju dari sudut ekonomi ialah kadar embriogenik yang rendah.

Pada masa ini, kebanyakan makmal kultur tisu tempatan melaporkan kadar embriogenesis dalam lingkungan 6%. Oleh kerana pengetahuan yang terhad mengenai embriogenesis somatik kelapa sawit, maka proses tersebut sukar difahami, apatah lagi untuk memperbaiki tarafnya. Justru itu, kajian ini telah disesuaikan untuk memahami proses-proses asas yang mungkin berlaku semasa embriogenesis dengan menganalisa perbezaan di antara kultur *in vitro* kelapa sawit yang embriogenik (EC) dan tak-embriogenik (NEC).

Kajian awalan menumpukan kepada penjelasan tentang perbezaan antara EC dan

NEC pada peringkat mikroskopikal. Sebahagian besar proembrio (PE) boleh didapati pada EC. Fenomena di mana pengasingan sel-sel adalah syarat untuk embriogenesis, dapat diperhatikan semasa PE terbentuk. Hipotesis dibuat bahawa sel-sel pinggiran sekeliling setiap struktur PE mungkin melalui proses kematian sel yang telah dirancang ('Programmed Cell Death', PCD), yakni menghasilkan keadaan 'isolation of cells'. Hipotesis di mana PCD memainkan peranan yang penting dalam proses embriogenesis selanjutnya disokong oleh kajian-kajian di peringkat fisiologi dan molekular. EC didapati lebih aktif secara metabolik jika dibandingkan dengan NEC, oleh itu EC memerlukan satu sistem yang efisien untuk mengatasi pengumpulan spesies oksigen reaktif ('Reactive Oxygen Species', ROS) iaitu hasil sampingan toksik daripada metabolisme aerobik. Adalah dipercayai bahawa OPEm1 yang khusus kepada tisu embriogenik, yang mengekod untuk sejenis antioksidan yang dikenali sebagai 'peroxiredoxin', mungkin bertindak dengan cara melindungi proembrio terhadap kerosakan akibat tindakan ROS tetapi membiarkan sel-sel disekelilingnya mati.

OPEm1 merupakan 'peroxiredoxin' yang pertama yang telah dipencilkan daripada palma dan ia berupaya untuk dieksploitasikan sebagai petanda molekular untuk mengesan keupayaan embriogenik dalam kultur *in vitro*. Tambahan pula, dengan pengetahuan keadaan fisiologi kultur embriogenik dan tak-embriogenik, satu kaedah untuk mengesan keupayaan embriogenik tanpa mengorbankan kultur dapat diperkembangkan dengan menggunakan mekanisme tindakbalas pewarna oksidatif dalam kultur media.

Selain daripada itu, pemencilan gen embriogenik yang lain juga telah dijalankan dengan menggunakan kaedah pengklonan segera untuk cDNA yang mempunyai pengekspresan yang berbeza. Teknik ini lebih dikenali sebagai 'Suppression Subtractive Hybridization'. Daripada jumlah klon sebanyak 595 yang telah disaring, hanya 66 didapati tisu embriogenik terkhusus. Di antara klon-klon tersebut, satu daripadanya telah diuraikan dan klon tersebut didapati berkait rapat dengan 'class IV chitinase EP3'. Klon ini telah dinamakan sebagai OPSSH1. Terdapat bukti daripada penganalisaan Northern yang mencadangkan bahawa terdapat beberapa subset 'class IV chitinase EP3' yang berlainan telah dikesan dari pemerhatian dua transkrip yang berlainan saiz. Kemungkinan transkrip-transkrip tersebut mengekod protein-protein yang berlainan fungsi. Oleh kerana penghasilan kebanyakan serpihan melalui kaedah ini adalah pendek, maka ianya terlalu awal untuk mencadangkan satu peranan fungsi kepada 'endochitinase' dalam sistem kelapa sawit.

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I certify that an Examination Committee met on 23rd March 2001 to conduct the final examination of Ong Li Mei on her Doctor of Philosophy thesis entitled “An Examination of Embryogenic and Non-Embryogenic Cultures of Oil Palm (*Elaeis guineensis* Jacq.)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Norihan Salleh, Ph.D.
Department of Biotechnology,
Faculty of Food Science and Biotechnology,
Universiti Putra Malaysia
(Chairperson)

Harikrishna Kulaveerasingam, Ph.D.
Associate Professor,
Genome Centre,
Institute of Bioscience,
Universiti Putra Malaysia
(Member)

Tan Siang Hee, Ph.D.
Genome Centre,
Institute of Bioscience,
Universiti Putra Malaysia
(Member)

Ruslan bin Abdullah, Ph.D.
Associate Professor,
School of Bioscience and Biotechnology,
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia
(Member)

Keith Lindsey, Ph.D.
Professor,
Department of Biological Sciences,
University of Durham DH1 3LE,
United Kingdom
(Independent Examiner)



MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor/Deputy Dean of Graduate School
Universiti Putra Malaysia

Date: 04 APR 2001

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor
Deputy Dean of Graduate School
Universiti Putra Malaysia

Date:

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



(ONG LI MEI)

Date: 4/4/2001

TABLE OF CONTENTS

DEDICATION.....	Page ii
ABSTRACT.....	iii
ABSTRAK.....	vi
ACKNOWLEDGEMENTS.....	ix
APPROVAL SHEETS.....	xi
DECLARATION FORM.....	xiii
LIST OF TABLES.....	xvii
LIST OF FIGURES.....	xviii
LIST OF ABBREVIATIONS.....	xx

CHAPTER

1	INTRODUCTION.....	1
2	LITERATURE REVIEW.....	4
	2.1 Significance of Clonal Propagation.....	4
	2.2 Tissue Culture of the Oil Palm.....	5
	2.3 Embryogenesis.....	8
	2.3.1 The Oil Palm Embryo.....	12
	2.3.2 Cell Division and Pattern Formation in Embryogenesis.....	13
	2.3.3 Patterning of the Embryo Body.....	16
	2.4 Zygotic Embryogenesis versus Somatic Embryogenesis	18
	2.5 Embryogenesis versus Organogenesis.....	23
	2.6 Embryogenic Calli (EC) versus Non-embryogenic Calli (NEC).....	25
	2.7 Mechanisms of Somatic Embryogenesis.....	29
	2.7.1 Physiological and Biochemical Aspects.....	30
	2.7.2 Molecular Biology of Embryogenesis.....	37
3	MATERIALS AND METHODS.....	43
	3.1 Plant Materials.....	43
	3.1.1 Tissue Cultured Materials.....	43
	3.1.2 Zygotic Embryos.....	44
	3.1.3 Gaseous Study of Oil Palm <i>in vitro</i> Cultures.....	44
	3.2 Microscopy Experiments.....	44
	3.2.1 Histology.....	44
	3.2.2 Scanning Electron Microscopy.....	46
	3.2.3 Transmission Electron Microscopy.....	46
	3.3 Physiological Experiment.....	48
	3.4 Analysis of Proteins.....	51
	3.4.1 Isolation of Total Proteins.....	51
	3.4.2 Protein Quantification.....	52
	3.4.3 SDS-PAGE Gel Electrophoresis.....	53
	3.4.4 Staining of Polyacrylamide Gels.....	56

3.5	Molecular Experiments.....	58
3.5.1	Extraction of Total and Poly A ⁺ RNA.....	58
3.5.2	Construction of the 15-week Zygotic Embryo cDNA Library.....	61
3.5.3	Screening of the Library.....	64
3.5.4	Construction of an Embryogenic Enriched Library.....	69
3.5.5	Reverse Transcription PCR (RT-PCR).....	74
3.5.6	Blotting Procedure.....	75
3.5.7	Sequence Analysis.....	78
4	RESULTS	80
4.1	Microscopical Analyses of Embryogenic (EC) and Non- embryogenic (NEC) Cultures.....	80
4.1.1	Histological Examination.....	80
4.1.2	Scanning Electron Microscopical Examination.....	82
4.1.3	Ultrastructural Examination with the Transmission Electron Microscope.....	82
4.2	Physiological and Protein Analyses: EC versus NEC... ..	87
4.2.1	Physiological Examination of the Cultures.....	87
4.2.2	Comparison of Protein Profiles Between EC and NEC.....	92
4.3	Isolation of cDNA Clones from the Libraries Constructed.....	95
4.3.1	Screening of the Zygotic Embryo cDNA Library with Heterologous Probes.....	95
4.3.2	Isolation of Embryogenic Related Genes via Suppression Subtractive Hybridization (SSH)... ..	96
4.4	The Identification of OPEm1: A Member of a Novel Class of Peroxidase.....	97
5	DISCUSSION.....	110
5.1	What are the Differences Observed Between EC and NEC?.....	110
5.2	Hormonal Regulation During Embryogenesis.....	119
5.3	Is Cell Isolation Caused by Programmed Cell Death (PCD)?.....	127
5.4	Isolation of Clones of Interest from Libraries.....	130
5.5	OPEm1, a Potential Embryogenic Marker for Oil Palm <i>in vitro</i> Cultures.....	137
6	CONCLUSION.....	142
	BIBLIOGRAPHY.....	145
	APPENDICES.....	170
	Appendix A: Basal Medium for M9 and M11.....	170
	Appendix B: Physiological Examination Data of the Cultures.....	172
	Appendix C: Formulation for Media and Solutions.....	173
	Appendix D: Preparation of Host Strains.....	174

LIST OF TABLES

Table		Page
4.1	A summarized result of a pairwise comparison between EC 9, EC 11, NEC 9, NEC 11 and SC.....	90

LIST OF FIGURES

Figure	Page
2.1 The oil palm tissue culture process.....	6
2.2 An overview of plant embryogenesis.....	11
2.3 A schematic representation of the oil palm seed and growth of seedling with examples of oil palm fruits and seed.....	14
2.4 The cell cycle and its interconnection with the growth control pathway.	39
3.1 Schematic diagram of the set-up for physiological analysis of <i>in vitro</i> cultures.....	50
4.1 Oil palm cultures derived from leaf explants (<i>le</i>) grown on solidified callus initiation medium.....	81
4.2 Histological sections of embryogenic and non-embryogenic materials.....	83
4.3 Scanning electron micrographs of embryogenic, non-embryogenic and zygotic embryo materials.....	84
4.4 Ultrastructures of embryogenic and non-embryogenic cultures.....	85
4.5 Ultrastructures of embryogenic materials.....	86
4.6 Transmission electron micrograph of cells in embryogenic calli.....	87
4.7 Carbon biochemistry of growth and respiration.....	89
4.8 Graph depicting the production of CO ₂ (ml) through respiration against fresh weight of the cultures (x10 ⁻⁴ kg).....	92
4.9 Protein analysis of embryogenic and non-embryogenic cultures.....	94
4.10 Analysis of OPSSH1.....	98
4.11 Sequence alignment of OPSSH1 with other members of the endochitinase proteins.....	99
4.12 Reverse transcription of zygotic embryo total RNA with AGL15AtF and AGL15AtR.....	100
4.13 Nucleotide and deduced amino acids sequences of OPEm1.....	102

4.14	Analysis of OPEm1	104
4.15	3-D structure of Peroxiredoxin and OPEm1	107
4.16	Sequence alignment of OPEm1 with examples of 1-Cys and 2-Cys peroxiredoxins.....	108
4.17	Sequence alignment of OPEm1 with examples of other members of 1- Cys peroxiredoxin.....	109
5.1	MTT is reduced to monoformazan (purple) by the reducing agent NAD(P)H.....	118
5.2	Developmental phases of somatic embryogenesis in carrot suspension cultures.....	121
5.3	A schematic representation of the SSH method.....	133

LIST OF ABBREVIATIONS

α	alpha
β	beta
λ	lambda
%	percentage
°C	degree centigrade
Å	angstrom
Arg	arginine
2-BE	ethyleneglycolmonobutylether
bp	base pair
BDMA	n-benzyl dimethyl amine
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
Ci	curie
CO ₂	carbon dioxide
C-terminal	carboxyl terminal
Cys	cysteine
1-D	one dimensional
2-D	two dimensional
2,4-D	2,4- dichlorophenoxy acetic acid
DDSA	dodecenyl succinic anhydride
DNA	deoxyribonucleic acid
DNase 1	deoxyribonuclease 1

cDNA	complementary DNA
dNTPs	Deoxynucleotides
dATP	2'-deoxy-adenosine-5'-triphosphate
dCTP	2'- deoxy-cytidine-5'-triphosphate
dGTP	2'- deoxy-guanosine-5'-triphosphate
dTTP	thymidine-5'-triphosphate
dH ₂ O	distilled water
DEPC	diethyl pyrocarbonate
DMF	dimethyl fluoride
DMSO	dimethylsulphonyl oxide
DTT	dithiothreitol
D X P	<i>dura x pisifera</i>
EtBr	ethidium bromide
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol bis- (β-aminoethyle ether)
g	gram
HCl	hydrochloric acid
His	histidine
H ₂ O ₂	hydrogen peroxide
hr	hours
IPTG	isopropyl-β-D-thiogalactoside
Jacq.	Jacquin
LB	luria-bertani
k	kilo

kb	kilobase
KCl	potassium chloride
kDa	kilodalton
L	liter
LiCl	lithium chloride
M	molar
mA	milliampere
mg	milligram
min	minute
ml	milliliter
mm	millimeter
mM	millimolar
mmol	millimole
MMLV	Maurine Moloney Leukemia Virus
MgCl₂	magnesium chloride
MgSO₄	magnesium sulphate
MNA	methyl nadic anhydride
MOPS	3-(N-morpholino) propane-sulphonic acid
MPOB	Malaysian Palm Oil Board
mRNA	messenger RNA
MW	molecular weight
N	normal
NAA	napthylacetic acid
NaCl	sodium chloride

NaOAc	sodium acetate
ng	nanogram
NO	nitrite oxide
N-terminal	amino terminal
OD	Optical density
ORF	open reading frame
OPZE	oil palm zygotic embryo
OPEm	oil palm embryogenic
OPSSH	oil palm suppression subtractive hybridization
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pfu	plaque forming unit
pI	isoelectric point
Poly A ⁺ RNA	polyadenylated RNA
PVP	Polyvinylpyrrolidone
PVPP	Polypolyvinylpyrrolidone
RNA	ribonucleic acid
rRNA	ribosomal RNA
RNase	ribonuclease
rpm	revolution per minute
RT	reverse transcriptase
SDS	sodium dodecyl sulfate
TAE	tris acetate EDTA
TBE	tris borate EDTA

TEMED	N,N,N',N'-tetramethylethylenediamine
U	unit
μg	microgram
μl	microliter
μm	micrometer
UPM	Universiti Putra Malaysia
UV	ultraviolet
v/v	volume per volume
WAA	weeks after anthesis
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranose